REMARKS

In view of the following remarks, the Examiner is requested to allow claims 1-15, the only claims pending and under examination in this application.

The specification has been amended at paragraph [0014] to recite the art recognized full name for CCF2.

Claims 1, 6, 11, 12 and 15 are amended.

Support for the amendments to claims 1 and 12 is found in the specification at, for example, paragraph [0021] and in Figure 1.

Claim 6 has been amended to recite the full name corresponding to the abbreviation CCF2. Support for this amendment can be found in the specification, particularly at paragraph [0014] as amended.

Claims 11 and 15 have been amended to correct the spelling of *pseudotyped* at line 2 of each claim.

No new matter has been added.

CLAIM OBJECTIONS

Claim 6

Claim 6 was objected to for not reciting the full name of the substrate corresponding to the abbreviation CCF2.

Present Claim 6 recites the full name of CCF2 as "coumarin cephalosporin fluorescein". The specification is also amended at paragraph [0014] to indicate the full name of CCF2.

The structure of CCF2, which was designed and synthesized by Zlokarnik et al. in 1998 (document is of record), was known at the time of filing of the priority application of the instant case. Zlokarnik et al. does not provide a full name for CCF2. Nevertheless, it is thus clear from Zlokarnik et al. that one of ordinary skill would understand the compound to which "CCF2" refers (see, e.g., Zlokarnik et al., Fig. 1). Later publications disclose that CCF2 stands for coumarin cephalosporin fluorescein. For example, Knapp T et al. *Cytometry A* **2003**, *51*:68-78 (Exhibit A), on which Zlokarnik is a co-author, indicates the full name of CCF2 in the abstract. The nomenclature "/AM" refers to a cell-permeant form of CCF2 (see the specification at, for example, paragraph [0084]). It is evident from the use of the term in claim 6 that the form of CCF2 that is substantially not cell membrane permeable is

intended. Campbell R *Trends Biotech* **2004**, *22*:208-11 also indicates that the full name of CCF2 is coumarin cephalosporin fluorescein, and tha "/AM" refers to the acetoxymethyl ester of CCF2 (Exhibit B, see page 209, second column, last paragraph).

Therefore, it was recognized in the art that CCF2 is the same substrate as coumarin cephalosporin fluorescein. Therefore, the amendments to claim 6 and to the specification do not introduce new matter.

Withdrawal of this objection is respectfully requested.

Claims 11 and 15

Claims 11 and 15 were objected to for the misspelling of *pseudotyped.* Present Claims 11 and 15 recite the correct spelling. These objections may be withdrawn.

CLAIM REJECTIONS – 35 U.S.C. § 103

Claims 1-10 and 12-14 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Muthumani et al. (DNA and Cell Biol 2000, 19:179-88) ("Muthumani") in view of Zlokarnik et al. (Science 1998:279:84-88) ("Zlokarnik"). This rejection is respectfully traversed.

The Examiner asserted that Muthumani discloses a method for detecting fusion of an enveloped retrovirus, such as HIV, wherein the chimeric virion contains Viral protein R (Vpr) from HIV operably linked to the reported protein Green Fluorescent Protein (GFP). The Examiner acknowledged that Muthumani does not disclose using beta-lactamase and CCF2 as a detection signal for the virion-based fusion assay, and cited Zlokarnik in an effort to remedy the deficiency in Muthumani. The Examiner then concluded that it would have been obvious to substitute the GFP of Muthumani with the beta-lactamase and CCF2 of Zlokarnik.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation either in the cited references themselves or in the knowledge generally available to an art worker, to modify the reference or to combine reference teachings so as to arrive at the claimed method. Second, the art must provide a reasonable expectation of success. Finally, the prior art reference must teach or suggest all the claim limitations (MPEP § 2143). The teaching or suggestion to arrive at the claimed method and the reasonable expectation of success must both be found in the prior art, not in Applicant's disclosure (MPEP § 2143 citing with favor, *In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

In fact, contrary to the Examiner's assertion, Muthumani does not disclose detection of virion fusion where the detectable signal is not detectable prior to a complete viral fusion event, which is characterized by intracellular delivery of the contents of the viral capsid as required by the present claims. As illustrated in Exhibits A and B, enveloped virions can enter cells by two mechanisms: fusion (Exhibit A[1]) and endocytosis (Exhibit B). Fusion events involve not only association with the cell, but also *fusion* of the viral envelope with the host cell membrane. As illustrated in Exhibit A, this results in delivery of the contents of the virion into the host cell cytoplasm. In contrast, in endocytosis (Exhibit B) no fusion event occurs. Instead the viral envelope remains intact, and the contents of the virion do not come into contact with the intracellular milieu of the host cell.

If one used the Vpr-GFP of Muthaumani, one could not distinguish between these two pathways. Vpr-GFP epifluorescence is readily detected regardless of whether the virion has fused and has released the Vpr-GFP protein into the cytoplasm, or alternatively whether the whole virion has been endocytosed into the cell with the virion remaining intact, but sequestered in an internal vesicle. Indeed in some cells, endocytosis can account for more than 90% of the uptake of virions. In sharp contrast to a *bona fide* fusion pathway, productive infection of the target cell does not occur after endocytosis because the virion is ultimately degraded in the internalized vesicle. Therefore, one cannot conclude reasonably from Muthumani that virion fusion is either being detected or being detected independently of endocytosis.

In contrast, Applicants' invention involves the use of a chimeric viral protein contained in an enveloped retroviral virion. The chimeric viral protein comprises a reporter polypeptide (e.g., BlaM) operably joined to a viral accessory protein (e.g., Vpr), wherein the reporter polypeptide provides a detectable signal upon intracellular delivery of the chimeric viral protein into the target cell cytoplasm, and wherein the detectable signal is not detectable prior to said intracellular delivery into the target cell cytoplasm. The claimed method thus permits the selective study of viral entry into the host cell via the fusion pathway.

For example, in one embodiment of Applicants' invention, the reporter polypeptide portion of the chimeric viral protein is beta-lactamase (BlaM) and the substrate is CCF2. Endocytosis of virions does not scored as a viral fusion event in Applicants' assay because the CCF2 substrate molecule is not able to cross the membrane forming either the endocytic vesicle or the membrane of the intact virion,

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[1] Exhibit A is a copy of Figure 1 of the present application.

both of which would be needed for CCF2 to access the BlaM-Vpr fusion protein and for CCF2 to be cleaved by BlaM (see Exhibit B).[2]

As recited in Applicants' claims, a detectable signal is produced only when the reporter polypeptide cleaves the substrate within the target cell, and not prior to this event. This should be contrasted with the Vpr-GFP, wherein (as pointed out *supra*) epifluorescence would be readily detected regardless of whether the virion has fused and has released the Vpr-GFP protein into the cytoplasm or alternatively whether the whole virion has been endocytosed into the cell, with the virion remaining intact but sequestered in an internal vesicle.

Therefore, nothing in the combination of documents teaches or suggests modifying the process of Muthumani to make the process a selective process for detecting fusion of an enveloped retrovirus to a target cell. Motivation to do so comes only from Applicants' specification, which of course would represent impermissible hindsight.

Accordingly, for at least the reason that there would have been no motivation in the cited documents to combine the documents in order to reach Applicants' invention, there is no *prima facie* obviousness. Withdrawal of this rejection is respectfully requested.

Claims 11 and 15 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Muthumani in view of Zlokarnik, as applied in the above rejection, and further in view of Miyanohara et al. (U.S. Patent 5,739,018) ("the '018 patent"). This rejection is respectfully traversed.

The '018 patent was cited only for its disclosure of a pseudotyped virion. As such, the '018 patent does not overcome the deficiencies detailed above in the combination of Muthumani in view of Zlokarnik. Specifically, the '018 patent does not teach or suggest modifying the process of Muthumani to make the process a selective process for detecting fusion of an enveloped retrovirus to a target cell.

Accordingly, for at least the reason that there would have been no motivation in the cited documents to combine the documents in order to reach Applicants' invention, there is no *prima facie* obviousness. Withdrawal of this rejection is respectfully requested.

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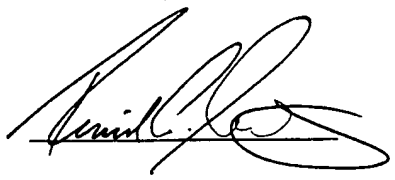
[2] As described in the specification, CCF2 is provided in the host cells by loading the cells with the membrane-permeable form, CCF2/AM, which undergoes de-esterification by non-specific cytoplasmic esterases in the cell cytoplasm to produce -membrane permeable CCF2, which is largely trapped within cells.

CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

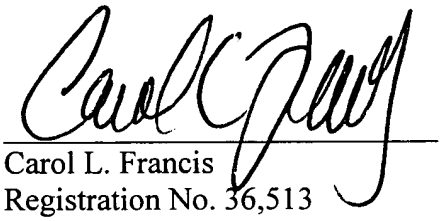
The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number UCAL-283.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

By: 

Date: October 10, 2006

Richard A. Schwartz
Registration No. 48,105

By: 

Date: October 10, 2006

Carol L. Francis
Registration No. 36,513

Enclosures:

Exhibit A:	Knapp T et al. *Cytometry A* **2003**, *51*:68-78
Exhibit B:	Campbell R *Trends Biotech* **2004**, *22*:208-11
Exhibit C:	Schematic Illustrating Viral Fusion
Exhibit D:	Schematic Illustrating Endocytosis Of A Virus

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